REMARKS

Claims 1, 3, 5, 9, and 12-14 are pending in this application and stand rejected. Reconsideration is requested in view of the following remarks.

Claims 1 and 5 have been amended. Basis for these amendments appear in the specification on page 5, lines 4–11 and page 5, line 29 – page 6, line 13; page 10, lines 23–26; page 15, lines 1–6 and 14 – 19; and page 23, lines 25-26.

Response to Claim Rejections Under 35 U. S. C. § 103

Claims 1, 3, 5, 9, and 12-14 were rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Garner *et al.* in view of Lord, Holm *et al.* Jennissen *et al* and Eigel *et al.*

The rejection alleges that Garner *et al.* disclose the transgenic production of fibrinogen in milk of animals. The rejection correctly indicates that the reference lacks any teaching of the use of HIC chromatography, and the feature of the invention that the recovered fibrinogen has high Aα-chain integrity. Garner *et al* only describe recovering fibrinogen from milk using "standard practices such as skimming, precipitation, filtration and protein chromatography techniques" (column 9, lines 23-25 of Garner *et al*). There is however, no teaching or suggestion of using the method applied in the claimed invention.

The deficiencies of Garner *et al.* are not remedied by the four secondary references. None related to the production of fibrinogen in milk. Moreover, the fact that no fewer than five references must be combined to support the rejection is itself evidence that the claimed invention is not obvious. *See, Adams v. United States*, 141 USPQ 361, 362 (US 1969).

The rejection alleges that Lord teaches recombinantly produced fibrinogen which can be collected and purified by any known method in the art, which is alleged to include HIC chromatography (col. 6, lines 36-53). The rejection alleges that Lord teaches the inclusion of protease inhibitors to prevent degradation of the recombinant product.

Lord does not teach a method as claimed for obtaining fibrinogen with high Aα chain integrity comprising an elution step which results in the selective removal of fibrinogen sub-fractions to produce high Aα chain integrity fibrinogen. Lord discuss the purification of fibrinogen from cell culture medium and not from milk. The purification can be through the use of known methods, as indicated in column 6, lines 38-44 of Lord *et al.* However, Lord is not

pertinent to the present invention because it does not describe a method of purifying fibrinogen from milk.

The deficiencies in Garner *et al.* and Lord are not remedied by Holm *et al.* or Jennissen *et al.*

The Examiner states that Holm *et al.* disclose the fractionation of fibrinogen. However, Holm *et al.* does not disclose the use of HIC to fractionate fibrinogen. The fibrinogen is first purified from plasma by precipitation with beta-alanine in the presence of citrate and protease inhibitors. Stepwise precipitation with ammonium sulphate was then used to obtain fractions with a high degree of purity. This work is cited in the present application (see page 8, lines 18-20). The precipitation techniques of the present application are for a different purpose – for the separation of the fibrinogen product away from the damaging protease enzymes (see page 10, lines 9-17). The HIC step is then used to select for a fibrinogen product which could comprise up to 100% fibrinogen. This is not described in Holm *et al.*

The Examiner states that Holm *et al.* compare the three fibrinogen sub-fractions (F1, F2 and F3) and note that the high molecular weight fraction (F1) contained intact Aα chains and no observable chain remnants, as compared to the low molecular weight fractions where degradation of the Aα chains is clearly evident. Holm *et al.* merely analyze the integrity of the fibrinogen sub-fractions. The present invention on the other hand provides a method by which the integrity of the resulting fibrinogen can be controlled through selective elution of particular sub-fractions. Holm *et al.* do not provide for the selective elution of sub-fractions, or the isolation of high Aα-chain integrity fibrinogen.

The Examiner maintains that Jennissen *et al.* disclose the use of HIC to produce fibrinogen that was molecularly uniform, fully active and had a molecular weigh of 72 kDa. The Examiner thus alleges that Jennissen *et al.* demonstrate that HIC may be used to purify fibrinogen. In the previous Office Action, the Examiner maintained that fibrinogen molecules with a molecular weigh of 72 kDa are intact. However, the document does not disclose the use of HIC to resolve the fibrinogen sub-fractions, or subsequent step-wise elution to select those sub-fractions which are to comprise the product. Native biologically active fibrinogen is purified

away from plasma in a single step in Jennissen *et al.* Plasma is applied to an HIC column, yielding purified fibrinogen.

According to the present invention, HIC chromatography is used as a fractionation technique for resolving fibrinogen sub-fractions. HIC further separates the fibrinogen from contaminants, and therefore the HIC step performs a second role, by resolving the fibrinogen sub-fractions. This use of HIC is neither taught nor suggested by Jennissen *et al.*

Eigel *et al.* is cited as disclosing that "plasmin was found in bovine milk and found to be identical to fibrinogen obtained from plasma". This is incorrect. While Eigel *et al.* provide evidence for the existence of plasminogen in milk and the formation of plasmin in milk, they do not state that the plasminogen found is "identical to fibrinogen obtained from plasma". Rather the Eigel *et al.* abstract concludes that bovine plasmin is identical to "alkaline milk protease". The latter is not fibrinogen. In any event, Eigel *et al.* adds nothing of pertinence to the patentability of the claimed invention.

According to the present invention, fibrinogen is precipitated from milk, and the precipitated fibrinogen is separated from protease enzymes. This results in the recovery of part-purified fibrinogen (claim 1). Alternatively, milk derived from a transgenic animal is provided (claim 5). The part-purified fibrinogen or the transgenic animal milk is then contacted with a hydrophobic interaction chromatography (HIC) resin under conditions wherein the fibrinogen binds to the resin. Removal of the bound fibrinogen from the column by elution results in the selective removal of fibrinogen sub-fractions to produce high Aα-chain integrity.

It would not have been obvious to one of ordinary skill in the art from the asserted references to combine the use of precipitation and HIC to purify fibrinogen and produce fibrinogen with high Aα chain integrity as defined by claims 1 and 5 Only Garner *et al.* disclose production of fibrinogen in milk, and does not disclose isolation of fibrinogen with HIC. The remaining asserted prior art, with the exception of Eigel *et al.*, deals with isolation of plasma-derived fibrinogen. There are significant differences between plasma-derived fibrinogen and milk-derived fibrinogen which render the isolation of the latter according to the present invention nonobvious over the asserted prior art. Those differences were identified in applicants' previous response, and are repeated herein:

(1) Plasma-derived fibrinogen does not contain all of the fibrinogen degradation products which occur in milk-derived fibrinogen as a result of the action of proteases in milk.

(2) Milk contains large amounts of casein, which comprises hydrophobic proteins that would be expected to disrupt the purification of fibrinogen from milk using HIC.

Even if assuming *arguendo* that would have been possible from the cited references to produce high Aα chain integrity fibrinogen from plasma, it would not have been obvious that HIC could be used on milk-derived fibrinogen for this purpose

The nonobviousness of using HIC to produce high Aα chain integrity fibrinogen from milk is further apparent when one considers the results of Wolling *et al.* As discussed in the applicants' previous response, Wolling *et al.* found that HIC could not be used to separate intact fibrinogen from degradation products.

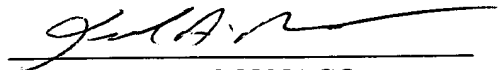
It would not have been obvious to one of ordinary skill in the art to use HIC to purify fibrinogen having high Aα-chain integrity from milk-derived fibrinogen, as provided by claims 1 and 5. Furthermore, for the reasons stated above, it would not have been obvious to one of ordinary skill in the art to combine the use of precipitation and HIC to produce fibrinogen with high Aα-chain integrity, as set forth in claim 1. Reconsideration and withdrawal of the Section 103 rejection is respectfully requested.

Conclusion

Applicants respectfully submit that all pending claims are in condition for allowance. An early action toward allowance is earnestly solicited. The Examiner is invited and encouraged to contact the undersigned attorney of record if such contact will advance examination and allowance of the application.

Respectfully submitted,

GRAHAM McCREATH, et al.

By: 

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